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# A New Locus for Autosomal Dominant Stargardt-Like Disease Maps to Chromosome 4

Marina Kniazeva,[1] Michael F. Chiang,[2] Basil Morgan,[2] Alfred L. Anduze,[4] Donald J. Zack,[2,3] Min Han,[1] and Kang Zhang[2]

[1]Department of Molecular, Cellular, and Developmental Biology, Howard Hughes Medical Institute, University of Colorado, Boulder; [2]Department of Ophthalmology, Wilmer Eye Institute, and [3]Departments of Molecular Biology and Genetics, and Neuroscience, Johns Hopkins University School of Medicine, Baltimore; and [4]Island Medical Center, Saint Croix, U.S. Virgin Islands

## Summary

**Stargardt disease (STGD) is the most common hereditary macular dystrophy and is characterized by decreased central vision, atrophy of the macula and underlying retinal-pigment epithelium, and frequent presence of prominent flecks in the posterior pole of the retina. STGD is most commonly inherited as an autosomal recessive trait, but many families have been described in which features of the disease are transmitted in an autosomal dominant manner. A recessive locus has been identified on chromosome 1p (STGD1), and dominant loci have been mapped to both chromosome 13q (STGD2) and chromosome 6q (STGD3). In this study, we describe a kindred with an autosomal dominant Stargardt-like phenotype. A genomewide search demonstrated linkage to a locus on chromosome 4p, with a maximum LOD score of 5.12 at a recombination fraction of .00, for marker D4S403. Analysis of extended haplotypes localized the disease gene to an ~12-cM interval between loci D4S1582 and D4S2397. Therefore, this kindred establishes a new dominant Stargardt-like locus, STGD4.**

## Introduction

Age-related macular degeneration (AMD) represents a heterogeneous group of retinal disorders and is the most common cause of irreversible blindness among elderly individuals in the United States (Bressler et al. 1988). Stargardt disease (STGD) shows some similarities to AMD. It is the most common hereditary macular dystrophy, with a prevalence of ~1/10,000. Clinically, STGD is characterized by decreased central vision during the first several decades of life, bilateral "bull's-eye" atrophy or a "beaten-bronze" appearance of the macula and underlying retinal-pigment epithelium, and frequent presence of prominent yellow "flavimaculatus flecks" in the posterior pole of the retina (Stargardt 1909; Hadden and Gass 1976; Noble and Carr 1979). Fluorescein angiography reveals a virtually pathognomonic "dark choroid" pattern (Fish et al. 1981). Electroretinography is normal early in the course of the disease but often shows mild to moderate abnormalities in late stages (Fishman 1976).

Classically, STGD is inherited in an autosomal recessive manner. A recessive locus has been mapped to chromosome 1p (STGD1 [MIM 248200]), and the corresponding gene on chromosome 1p has been cloned (Kaplan et al. 1993; Allikmets et al. 1997*b*). This gene encodes a retina-specific transmembrane protein, ABCR, which belongs to the ATP-binding cassette family of membrane transporters. It is of particular interest that mutations in the ABCR gene have been reported in a subset of patients with AMD (Allikmets et al. 1997*a*), although it should be noted that the interpretation of this finding is controversial (Dryja et al. 1998; Klaver et al. 1998; Stone et al. 1998). In addition to the more common recessive pattern, many families have been described in which features of STGD are transmitted in an autosomal dominant manner (Cibis et al. 1980; Lopez et al. 1990; Mansour 1992; Stone et al. 1994; Zhang et al. 1994). Dominant loci have been mapped to chromosome 13q (STGD2 [MIM 153900]) and to chromosome 6q (STGD3 [MIM 600110]) (Stone et al. 1994; Zhang et al. 1994).

In this report, we describe a kindred affected with an autosomal dominant form of STGD mapping to a new disease locus, on chromosome 4. In accordance with the guidelines of the HUGO Nomenclature Committee, the gene causing this disorder has been designated "STGD4." Linkage was established with a two-point maximum LOD score ($Z_{max}$) of 5.12 at $\theta = .00$, between

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Address for correspondence and reprints: Dr. Marina Kniazeva, Department of Molecular, Cellular, and Developmental Biology, Campus Box 347, University of Colorado, Boulder, CO 80309. E-mail: marinak@colorado.edu


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the disease and marker locus D4S403, and a 10-cM critical region between marker loci D4S1582 and D4S2397 was identified by analysis of extended haplotypes.

## Subjects and Methods

### *Subjects*

Informed consent was obtained from all participants, in accordance with the guidelines established by the Johns Hopkins University School of Medicine, Baltimore. Twenty-six members of a three-generation, nonconsanguineous Caribbean family were identified for study. A complete ophthalmic history was obtained, and an examination was performed on each subject. Individuals were considered to be affected on the basis of the presence of decreased visual acuity and bull's-eye or similar macular atrophy. Fluorescein angiography was done in four affected subjects, and two affected subjects underwent electroretinographic testing.

### *Genomic DNA Isolation and Genotype Analysis*

Blood was collected from family members by venipuncture, and genomic DNA was isolated with the QIAamp™ blood kit (Qiagen), according to the manufacturer's instructions. All DNA samples were analyzed with polymorphic short tandem repeat (STR) markers spanning 22 autosomes, with a step of ~24.2 cM (Research Genetics). PCR was performed according to the manufacturer's protocol (Research Genetics). In most cases, multiplex PCR was used. After amplification, PCR fragments were separated on a denaturing 6% polyacrylamide gel, and bands were visualized by exposure of the dry gel to x-ray film (Kodak).

### *Linkage Analysis*

Two-point and multipoint linkage analysis was conducted with the LINKAGE (version 5.1) package (Lathrop et al. 1985). Linkage analysis was done under conditions of no sex differences in recombination, complete disease penetrance, and disease-gene frequency of 1/10,000. Allele frequencies for the markers used were determined by the genotyping of 40 additional chromosomes. The reference genetic map used for linkage analysis was obtained from the Human Gene Map (Dib et al. 1996).

### *Haplotype Analysis*

Extended haplotypes of the individuals were constructed according to the order of STR markers in the Généthon genetic linkage map (Dib et al. 1996). Reconstruction of haplotypes was done, whenever possible, for individuals whose DNA samples were not available.

## Results

### *Clinical Examination*

Twelve of the 18 individuals at risk for inheriting the disease were found to be affected, on the basis of the presence of decreased visual acuity and macular atrophy (fig. 1*A*). Fluorescein angiography was done in four affected subjects, all of whom demonstrated the characteristic dark-choroid pattern of STGD. Three of the four fluorescein angiograms revealed typical STGD flavimaculatus flecks (fig. 1*B*). In addition, two affected subjects underwent electroretinographic testing. One of these individuals had a normal rod-photoreceptor response and a mildly decreased cone-photoreceptor response, and the other had a very mildly reduced rod response and a mildly reduced cone response (authors' unpublished

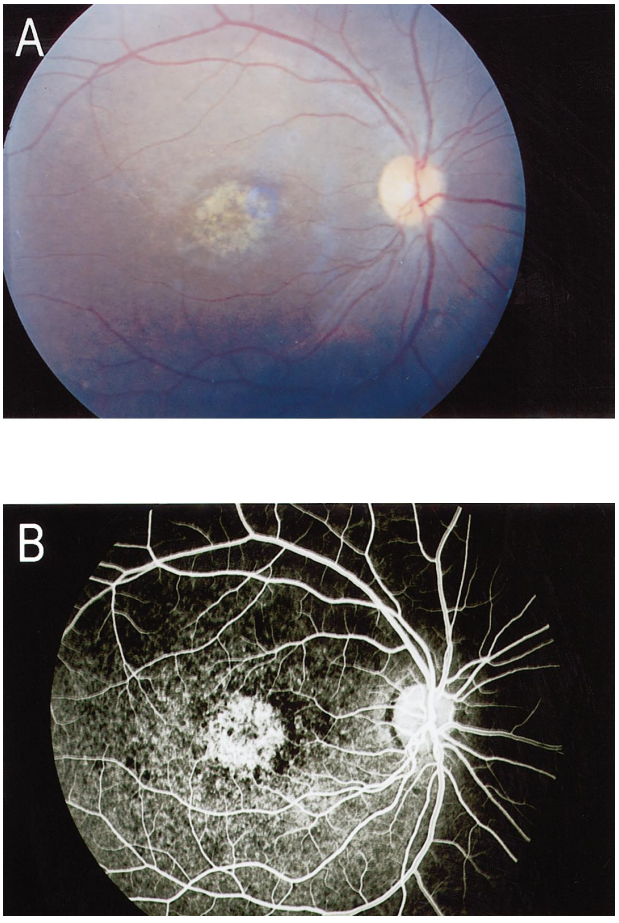


**Figure 1** Fundus photograph and fluorescein angiogram from right eye of patient III-13, a 33-year-old woman with decreased vision since age 15 years. Visual acuity was 20/400 in the right eye and 20/50 in the left eye. *A*, Fundus photograph showing bull's-eye macular atrophy. *B*, Fluorescein angiogram revealing dark-choroid pattern, as well as central retinal epithelial atrophy surrounded by numerous hyperfluorescent spots consistent with flavimaculatus flecks.

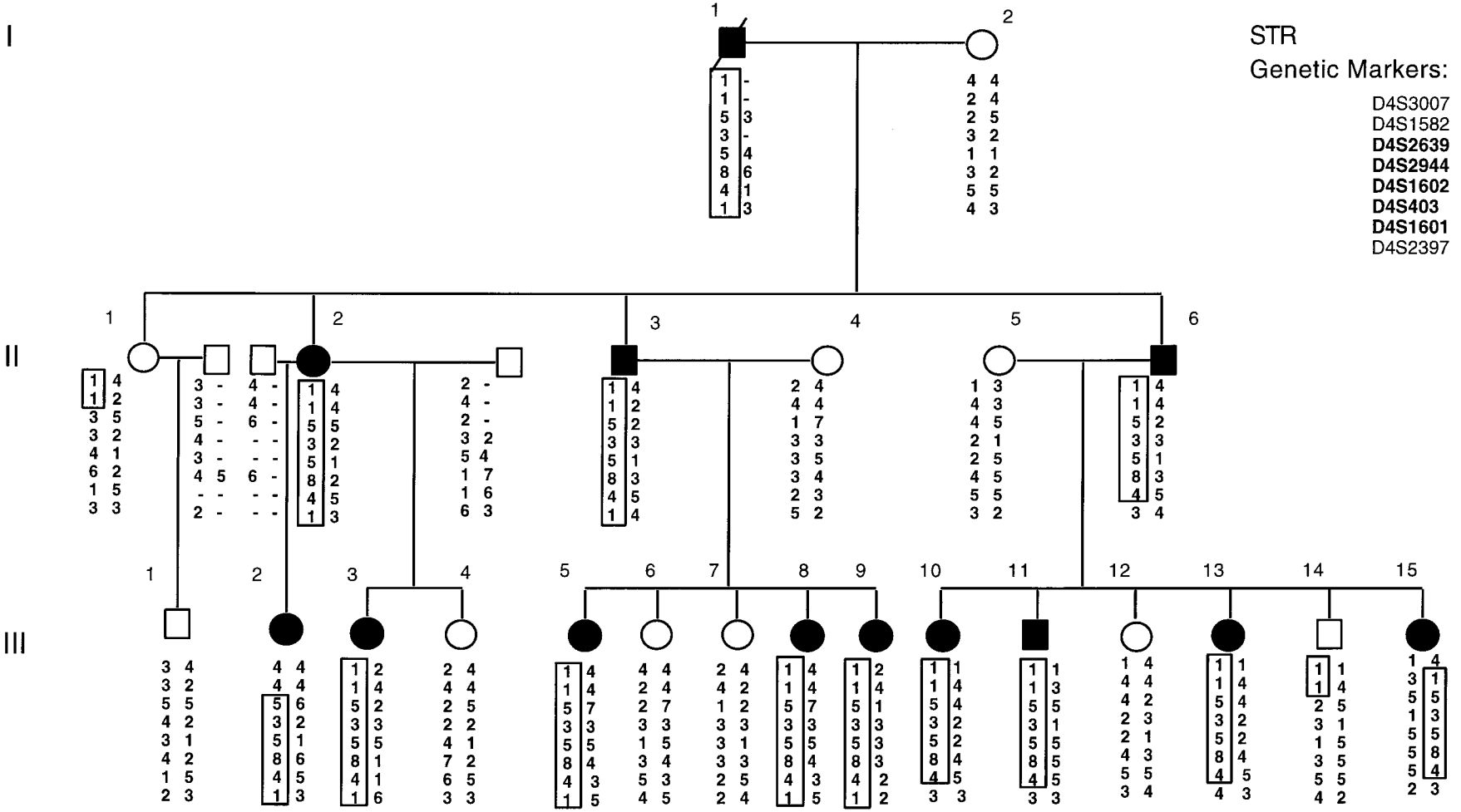


**Figure 2** Autosomal dominant Stargardt-like–disease pedigree. Boxes indicate the disease haplotypes. Squares denote males; circles denote females; blackened symbols denote affected individuals; unblackened symbols denote unaffected individuals; a diagonal slash through a symbol denotes that the individual is deceased; dashes (-) denote that data were not available.

**Table 1**

**Two-Point LOD Scores between Dominant Stargardt-Like Disease and DNA Markers**

| | LOD Score at $\theta$ = | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Marker | .00 | .01 | .06 | .11 | .16 | .21 | .26 | .31 | .36 | .41 |
| D4S3007 | $-\infty$ | -2.12 | .09 | .69 | .94 | 1.03 | 1.01 | .91 | .75 | .53 |
| D4S1582 | $-\infty$ | -.13 | 1.28 | 1.60 | 1.66 | 1.60 | 1.46 | 1.26 | 1.00 | .69 |
| D4S2639 | 4.21 | 4.15 | 3.81 | 3.45 | 3.08 | 2.68 | 2.26 | 1.81 | 1.35 | .87 |
| D4S2944 | .55 | .51 | .50 | .46 | .43 | .38 | .32 | .26 | .19 | .12 |
| D4S1602 | 4.82 | 4.74 | 4.36 | 3.96 | 3.53 | 3.08 | 2.60 | 2.09 | 1.56 | 1.012 |
| D4S403 | 5.12 | 5.04 | 4.63 | 4.20 | 3.76 | 3.28 | 2.77 | 2.23 | 1.67 | 1.08 |
| D4S1601 | 4.82 | 4.74 | 4.36 | 3.96 | 3.53 | 3.08 | 2.60 | 2.09 | 1.56 | 1.01 |
| D4S2397 | $-\infty$ | .16 | 1.42 | 1.64 | 1.65 | 1.56 | 1.39 | 1.18 | .93 | .63 |

data). A pedigree was constructed, with affected members of both sexes in each generation (fig. 2). Taken together, these data are consistent with autosomal dominant transmission of a Stargardt-like disease.

### *Genetic-Linkage Studies*

Polymorphic STR markers previously known to be linked to STGD, cone dystrophy (COD), and cone-rod dystrophy (CORD) were examined first. The analyzed loci included STGD2 on 13q34 (Zhang et al. 1994), STGD3 on 6q (Stone et al. 1994), COD3 (MIM 602093) on 6p21 (Payne et al. 1998), CORD2 (MIM 120970) on 19q13 (Evans et al. 1994; Freund et al. 1997; Swain et al. 1997), CORD5 (MIM 600977) on 17p (Small et al. 1996), and CORD6 (MIM 601777) on chromosome 17p12-p13 (Kelsell et al. 1997). No significant linkage was found to any of these loci.

A genomewide scan with 147 STR markers was performed, and linkage was found after 59% of the genome was excluded as a location of the disease gene. Two-point linkage analysis revealed a locus on chromosome 4, with $Z_{max} = 5.12$ at $\theta = .00$, for marker D4S403 (table 1). Linkage analysis was refined with 14 additional STR markers spanning a 32-cM region centered around marker D4S403. The LOD scores for loci D4S2639, D4S1602, and D4S1601 also satisfied the Morton (1955) criterion, reaching $Z_{max} > 4.0$ at $\theta = .00$. Multipoint linkage analysis (fig. 3) localized the disease interval to a region between markers D4S1582 and D4S2397, with 3-unit-LOD-score support interval ~10–22 cM to the right of marker D4S3007.

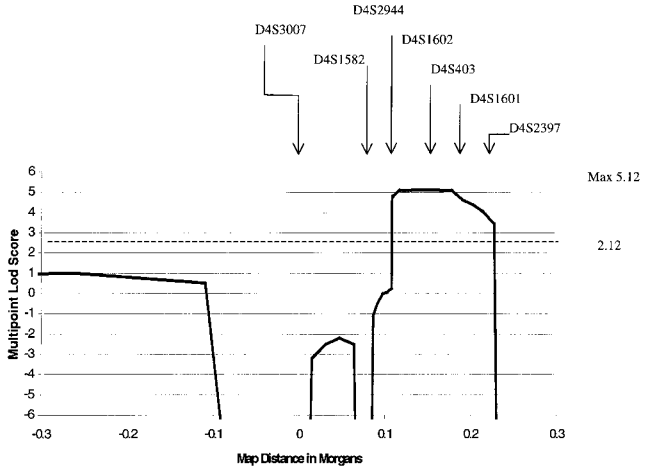


**Figure 3** Graph of multipoint LOD score, with genetic distance (in morgans) plotted versus the LOD score. The location of D4S3007 marker is considered to be at map position 0. The dotted line is drawn at $y = Z_{max} - 3$, to show a support interval for the maximum-likelihood estimate.

### *Haplotype Analysis*

Extended haplotypes were constructed on the basis of the following order of markers: D4S3007–D4S1582–D4S2639–D4S2944–D4S1602–D4S403–D4S1601–D4S2397 (fig. 2). These loci represent a region 12–32 cM from the top of the chromosome. The presumptive disease-associated haplotype was determined as a common extended haplotype for all affected individuals. Eight informative recombination events were identified in the family. On the basis of these results, the disease interval was localized between markers D4S1582 and D4S2397 on chromosome 4p. Within the region, no recombinations were detected at loci D4S2639, D4S2944, D4S1602, D4S403, and D4S1601.

## Discussion

STGD was originally described as an autosomal recessive trait (Stargardt 1909). STGD1, a chromosome 1p gene responsible for autosomal recessive STGD, has recently been cloned (Allikmets et al. 1997*b*). Autosomal dominant inheritance of a Stargardt-like phenotype is less common but has been described in a number of families (Cibis et al. 1980; Lopez et al. 1990; Mansour 1992; Stone et al. 1994; Zhang et al. 1994). Previous studies have identified two loci for dominant Stargardt-like disease: STGD2 and STGD3 (Stone et al. 1994;

Zhang et al. 1994). In the present study, we have performed genetic-linkage analysis of a kindred affected with autosomal dominant Stargardt-like disease. A genomewide search identified a chromosome 4 region containing the disease gene, with several STR markers cosegregating with the disease phenotype. A two-point $Z_{max}$ of 5.12 at $\theta = .00$ was obtained between the disease and marker locus D4S403. Multipoint analysis and analysis of extended haplotypes disclosed recombination events that restricted the disease interval to an ~12-cM region between markers D4S1582 and D4S2397. This defines a new locus, STGD4, for autosomal dominant Stargardt-like disease.

A candidate-gene search of a gene map of the human genome (Deloukas et al. 1998) identified two genes in this region that are expressed in the retina. The first gene encodes dihydropyrimidinase-related protein-1 (DRP-1) and has been mapped by Généthon by means of the radiation-hybrid panel. The biological function of DRP-1 is unknown, but two homologous proteins, DRP-2 and DRP-3, are thought to play a role in neuronal growth and maturation (Hamajima et al. 1996). The second gene encodes heat-shock protein 90 (HSP90 [MIM 140572]). It has been mapped by the Whitehead Institute for Biomedical Research/MIT Center for Genome Research by means of a YAC panel and belongs to a family of stress-inducible proteins (Rebbe et al. 1989). Heat-shock proteins are associated with various cellular signaling proteins and are believed to maintain tissue integrity against thermal, oxidative, and mechanical stresses. In particular, HSP90 has been shown to be expressed in rodents during visual-system development (Kojima et al. 1996).

The study of STGD and other hereditary macular dystrophies may have important implications for the study of AMD, which is the most common cause of irreversible blindness among elderly individuals in the United States. Historically, the genetic study of AMD has been limited by the clinical heterogeneity, late onset, and multifactorial etiology of the disease (Heiba et al. 1994; Seddon et al. 1996, 1997). STGD provides a useful model for the genetic and molecular study of macular degeneration, because of its numerous phenotypic and histopathological similarities to AMD, including progressively decreased vision, atrophy of the macula and retinal-pigment epithelium, dysfunction of retinal photoreceptors, and accumulation of debris within the retinal-pigment epithelium (Young 1987). In light of these similarities, it is reasonable to hypothesize that the genetic basis and pathogenesis of Stargardt-like macular dystrophy may be related to those of certain subsets of AMD. Consistent with this hypothesis is a recent report that certain ABCR sequence changes are found more commonly in persons with AMD (Allikmets et al. 1997*a*; van Driel et al. 1998), although these data remain controversial (Dryja et al. 1998; Klaver et al. 1998; Stone et al. 1998).

Cloning of the STGD4 gene, as well as further genetic and molecular study of hereditary macular dystrophies, should provide important insights into the pathogenesis of AMD. Compilation and analysis of other genes involved in macular dystrophies and AMD may eventually produce a more rational classification system for the various forms of macular degeneration. Finally, the discovery of specific genetic alterations in macular degeneration could create opportunities to improve clinical diagnosis and to offer more-effective, targeted therapies for patients.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr
Human Gene Map, http://www.ncbi.nlm.nih.gov/SCIENCE96
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for AMD, COD3 [MIM 602093], CORD2 [MIM 120970], CORD5 [MIM 600977], CORD6 [MIM 601777], HSP90 [MIM 140572], STGD1 [MIM 248200], STGD2 [MIM 153900], and STGD3 [MIM 600110])
Whitehead Institute, http://www.genome.wi.mit.edu (for genetic markers)

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